IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: LORENS et al.

Application No.: 09/935,124

Filed: August 21, 2001

For: MODULATORS OF ANGIOGENESIS

Customer No.: 20350

Confirmation No. 8377

Examiner: Maher M. Haddad, Ph.D.

Technology Center/Art Unit: 1644

DECLARATION OF DR. SACHA HOLLAND UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Sacha Holland, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I, Dr. Holland, am currently Associate Director for the Angiogenesis Program at Rigel Pharmaceuticals, Inc. I received my Bachelor of Arts with First Class Honors in natural sciences (specialization in genetics) from St John's College of the University of Cambridge in the United Kingdom in 1989. I received my Ph.D. in Molecular Biology from the University of London (UK) in 1994. I was a post doctoral fellow in the Laboratory of Dr. Tony Pawson at the Samuel Lunenfeld Research Institute of Toronto, Canada from 1994-2000. I joined the Rigel Pharmaceuticals Inc, as a scientist in 2000. I became a senior scientist and project leader in angiogenesis in 2003. In July 2005, I was appointed Associate Director and I

EXHIBIT A

am currently the Project leader for the Angiogenesis Program. A copy of my curriculum vitae is attached hereto as Exhibit E.

3. The present invention is a method for identifying a compound that modulates angiogenesis, by contacting the compound with a cell expressing an ILKAP polypeptide that has at least 90% identity to an amino acid sequence of SEQ ID NO:2.

4. I have read and am familiar with the contents of this patent application. In addition, I have read an Office Action and references cited therein, dated January 24, 2005, received in the present case. It is my understanding that the Examiner is concerned that the specification does not provide enablement of the full breadth of claimed invention. Specifically, the Examiner states that the neither the specification nor the art provide sufficient guidance to enable one of skill in the art to make and test for an anti-angiogenic compounds using any polypeptide having at least 90% identity to SEQ ID NO:2, without undue experimentation.

5. This declaration is provided to demonstrate that the specification teaches full use of the claimed methods. In particular, the specification in combination with the knowledge in the art at the time of filing provide sufficient guidance to allow one of skill to use the claimed methods to make and test for anti-angiogenic compounds, with only routine experimentation, using any ILKAP polypeptide having at least 90% identity to SEQ ID NO:2.

6. The nucleic acids encoding the recited ILKAP proteins were first disclosed in March 2001. Soon after, a reference was published disclosing that the ILKAP protein is a serine/threonine phosphatase. *See, e.g.*, Leung-Hagesteijn *et al.*, *EMBO J.*, 20:2160-2170 (2001), submitted as Exhibit B. The reference discloses sequence analysis and alignments between the ILKAP protein and previously identified members of the protein phosphatase 2C (PP2C) family, *e.g.*, Leung-Hagesteijn *et al.* at Figure 1B. The alignments identify conserved amino acid residues in the ILKAP protein. The reference provides experimental evidence of the function of the ILKAP protein by phosphatase assays, *e.g.*, Leung-Hagesteijn *et al.* at Figure 3A. The reference also confirms the sequence-based prediction of structure by demonstrating that

mutation of a conserved active site, DGH, identified through the sequence alignments, abolished the phosphatase activity of the ILKAP protein, *e.g.*, Leung-Hagesteijn *et al.* at page 2161 and Figure 3B.

The PP2C family of proteins is well conserved and well understood, as was known by those of skill in the art at the time of filing. Structures of PP2C family proteins had been determined. *See, e.g.*, Das *et al.*, *EMBO J.* 15:6798-6809 (1996), submitted as Exhibit C. The ILKAP protein of SEQ ID NO:2 has a high degree of identity with four conserved database domains based on PP2C proteins. *See, e.g.*, alignments of Exhibit D. For example, the catalytic domain of SEQ ID NO:2 is 99.6% aligned with the catalytic domain of cd0143, which has been publicly available since November 2000. The alignment identifies amino acid residues that are conserved between the ILKAP protein and cd0143. *See, e.g.*, alignments of Exhibit D.

7. Based on the experimental results and alignments of ILKAP disclosed in Leung-Hagesteijn *et al.* and alignments of the ILKAP amino acid sequence with PP2C conserved database domains, those of skill would be able to determine which ILKAP amino acids are most likely to require conservation to preserve function of the protein. Those of skill recognize that modification of conserved amino acid residues in a protein is more likely to affect protein activity than modification of non-conserved amino acid residues. Thus, non-conserved amino acid residues are more likely to tolerate modification or substitution. As is known in the art, amino acid substitutions can be based on amino acid residues found at corresponding positions in related proteins, *e.g.*, determined by sequence alignments. Moreover, certain amino acid substitutions are more likely to maintain protein activity and can be identified using tables that identify functionally similar amino acids and that are known in the art. In addition, computer programs that determine percent identity between two amino acid sequences are well-known in the art. Thus, using the alignments of the ILKAP protein with PP2C family members or with PP2C-based conserved database domains described above, and the knowledge in the art of amino acid relationships, those of skill can identify functional ILKAP proteins that have 90% or greater identity to SEQ ID NO:2 using, at most, routine experimentation. Moreover, using straightforward and routine mutagenesis and cloning techniques, *e.g.*, PCR based mutagenesis,

large numbers of variants of the ILKAP protein with 90% identity to SEQ ID NO:2 can be synthesized and assayed for ILKAP activity as disclosed in the specification.

8. The specification provides standard assays and working examples to identify a functional ILKAP polypeptide, for example *in vitro* assays, including phosphatase assays; cell based *in vivo* assays, such as cell migration assays, cell surface marker expression, *e.g.*, avb3 integrin cell proliferation, ILKAP expression, endothelial tube formation assays, haptotaxis assays; and animal based assays, such as chick CAM assays, mouse corneal assays, and effects on animal tumors. *See, e.g.*, specification at page 27, line 1 continuing to page 30, line 19; and Example 1, pages 44-45. The assays can be performed on multiple samples by, *e.g.*, a skilled laboratory technician. In addition, high through put assays can be performed using the assays disclosed in the specification. See, *e.g.*, specification at page 16, line 30 through page 18, line 21. Identification of functional ILKAP polypeptides with 90% identity to SEQ ID NO:2 is, therefore, well within the means of one of skill of the art with only routine experimentation, based on the disclosure of the specification and the knowledge of those of skill in the art at the time of filing.

9. I have read the references cited by the Office Action as supporting the rejection for alleged lack of enablement, *e.g.*, Atwood (2000), Skolnick and Fetrow (2000), and Metzler *et al.* (1997). I do not agree with the analysis of the Office Action. Atwood and Skolnick describe problems of predictive biology that do not apply to the claimed invention. Metzler *et al.*, in fact, supports the usefulness of sequence alignments for identifying amino acid residues that, if mutated, are most likely to affect protein activity. Metzler *et al.* also demonstrate that results of amino acid substitutions can be predicted by those of skill.

10. Both Atwood and Skolnick attempt to demonstrate that methods to identify a protein function based solely on comparison of an unknown amino acid sequence to known amino acid sequences with known functions are "unreliable" (Atwood) or "inadequate" (Skolnick). The Atwood and Skolnick references discuss only the problems of assigning function to a previously unknown protein based solely on sequence comparisons and lacking

experimental evidence of function. This type of analysis is not relevant to the claimed invention which recites use of ILKAP polypeptides. The specification and the information available at the time of filing provide experimental evidence of the function of the recited ILKAP proteins. The ILKAP protein has serine/threonine phosphatase activity, as disclosed in Leung-Hagesteijn *et al.*. Example 1 of the specification provides experimental evidence of the angiogenic activity if the ILKAP protein. As the function of the ILKAP protein has been experimentally determined, and assays to measure those function are known and routine, those of skill are able to identify functional variants of the ILKAP protein of SEQ ID NO:2. Thus, the concerns raised by Atwood and Skolnick do not apply to the claimed invention.

11. Metzler *et al.* disclose alteration of the activity of a human cytotoxic T lymphocyte-associated protein-4 (CTLA-4) through mutation of a conserved group of residues. At Figure 2, Metzler *et al.* provide an alignment of the amino acid sequences of 12 proteins in the CTAL-4/CD28 family. The authors noted that some residues of a conserved $M_{99}YPPPY_{104}$ domain are adjacent to a patch of conserved, charged residues on the A'GFCC' face of the protein. The authors hypothesized that the conserved charged residues could have an important function and would thus affect protein activity if modified.. The authors found that mutation of the conserved charged residues reduced binding activity of CTLA-4 to its ligands CD80 or CD86 as they had predicted. Thus, Metzler *et al.* demonstrate that generation of nonfunctional proteins by mutagenesis can be anticipated by those of skill, particularly when functional, structural and sequence information is known. In my opinion, Metzler *et al.* demonstrates that modification of proteins, including the ILKAP protein, can be done by those of skill with only routine experimentation.

12. In view of the foregoing, it is my scientific opinion that one of skill in the art would recognize how to make and use functional ILKAP polypeptides with 90% identity to SEQ ID NO:2 based on the information disclosed in the specification, as well as information in the art at the time of filing. In addition, it is my scientific opinion that any experimentation required by those of skill to identify such proteins would be routine. The specification, therefore, enables the invention.

Date: 10/27/05. By: Sacha Holland.

Sacha Holland, Ph.D.

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